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Award Number: W81XWH-05-2-0084

TITLE: Vulnerary Factors to Improve Bone Healing

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REPORT DATE: April 2007

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				<i>Form Approved</i> OMB No. 0704-0188	
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1. REPORT DATE (DD-MM-YYYY) 01-04-2007		2. REPORT TYPE Final		3. DATES COVERED (From - To) 15 Sep 2005 – 14 Mar 2007	
4. TITLE AND SUBTITLE Vulnerary Factors to Improve Bone Healing				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-05-2-0084	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jeffrey O. Hollinger, DDS, Ph.D. E-Mail: hollinge@cs.cmu.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Carnegie Mellon University Pittsburgh, PA 15213-3890				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES – original document contains color					
14. ABSTRACT No abstract provided.					
15. SUBJECT TERMS No subject terms provided					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 8	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Introduction

The objective for the work was to process rabbit bone specimens from the Institute of Surgical Research, followed by sectioning and staining of the samples.

No patents application were filed.

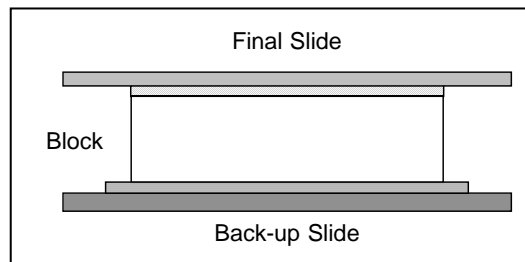
The rabbit bone samples were received fixed in 70% alcohol. Bone was cut longitudinally (Figure 1A) and cross-sectionally (Figure 2A) on a diamond band saw and thereafter processed (dehydrated and infiltrated with xylene) according to the following schedule:

1. 70% ETOH for 4 hours
2. 80% ETOH for 2 hours
3. 95% ETOH for 6 hours
4. Fresh 95% ETOH for 4 hours
5. 100% ETOH for 6 hours
6. Fresh 100% ETOH for 4 hours
7. Xylene for 6 hours
8. Fresh Xylene for 6 hours

Xylene was then removed by incubation in Methyl Methacrylate for 3 days. Penetration of Methyl Methacrylate into the sample was achieved by incubation in infiltration media (90% Methyl Methacrylate, 10% Dibutyl Phthalate, and 70% Benzoyl peroxide 1gm/100ml) for nine days, with a change of fresh solution every three days. Samples were embedded at room temperature in a solution containing: 90% Methyl Methacrylate, 10% Dibutyl Phthalate, and 2gm/100ml 70% Benzoyl peroxide. Once bones were embedded, the blocks were trimmed down using a diamond band saw.

In order to grind and polish the slides, the blocks are adhered to a “back-up slide” (25mm x 75mm x 2mm). Blocks are then ground and polished to an even smooth surface. The smooth surface of the block is then glued to a “final” slide (25mm x 75mm) - this glue is hardened with the use of an UV light. The “final slide” is then cut away from the “back-up” slide using the diamond band saw leaving about 200-300 micrometers of the embedded

The sample on
ground down
polished to a



sample on the “final” slide.
the “final” slide is then
to the desired thickness and
smooth even surface. The

slides are then acid etched to allow the stains to penetrate the tissue.

Acid etching of slides was performed using the following procedure:

1. Incubated in 1% Formic Acid for 3 minutes.
2. Washed well in running water.
3. Sonicated in 50% ETOH for 2 minutes.
4. Rinsed in water.

The slides were then air dried before staining took place. Once dried the slides were stained with Goldner's Trichrome and Toluidine Blue stains.

The Toluidine Blue staining of the slides was performed using the following procedure:

1. Incubated in Toluidine Blue for 15 seconds.
2. Rinsed well in de-ionized water.
3. Air dried.

The Goldner's trichrome staining of the slides was performed using the following procedure:

1. Incubated in Weigert's Iron Hematoxylin for 15 minutes.
2. Rinsed in distilled water.
3. Washed gently in running tap water for 15 minutes.
4. Rinsed in distilled water.
5. Incubated in Acid Fuchsin-Ponceau for 15 minutes.
6. Rinsed in two changes of 1% acetic acid.
7. Incubated in Molybdotophosphoric acid – Orange G for 8 minutes.

8. Staining was resolved in 2 changes of 1% acetic acid.
9. Incubated in Light Green SF Yellowish for 15 minutes.
10. Staining was resolved in 2 changes of 1% acetic acid.
11. Rinsed in water.
12. Air dried.

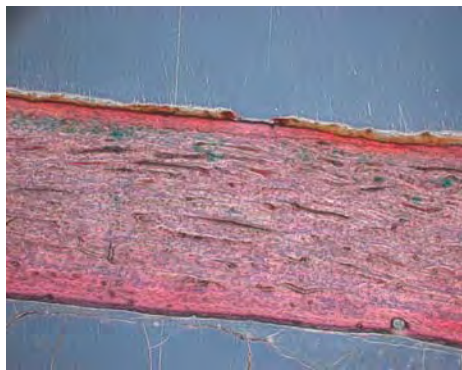
Figure 1

1A



Longitudinal section- fresh bone sample

1B



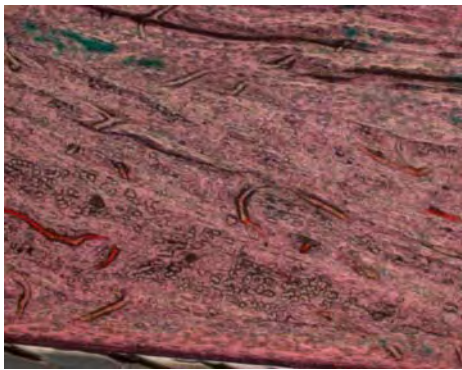
Trichrome- longitudinal section- 5X

1C



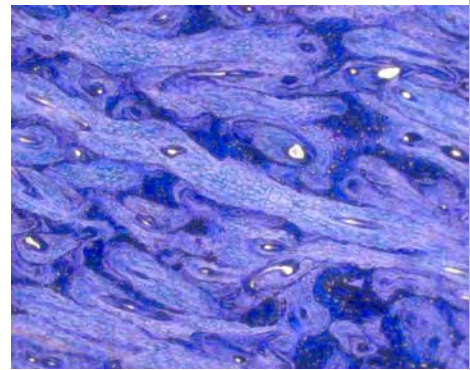
Toluidine Blue- longitudinal section- 5X

1D



Trichrome- longitudinal section- 10X

1E



Toluidine Blue-longitudinal section-10X

Figure 2

2A



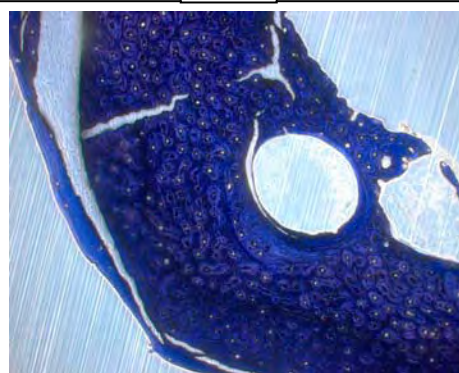
Cross-section- fresh bone sample

2B



Trichrome- cross-section- 5X

2C



Toluidine Blue- cross-section- 5X

2D



Trichrome- cross-section- 10X

2E



Toluidine Blue- cross-section-10X